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PICOPHYTOPLANKTONIC COMMUNITY STRUCTURE IN THE SUBTROPICAL PACIFIC OCEAN: A COMPARISON BETWEEN THE OFFSHORE AND COASTAL OCEAN AND CLOSED AND OPEN LAGOONS, IN RELATION WITH NITROGEN NUTRIENT AVAILABILITY

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ABSTRACT

Picophytoplanktonic community structure is composed of three components: *Prochlorococcus marinus* (Pro), *Synechococcus* spp. (Syn) and picoeukaryotes (Peu). The nitrogen nutrients $N_n = NO_3 + NO_2 + NH_4$ are one of the important controlling factors which determine community structure. In the open ocean, when the photic layer is N_n depleted ($N_n < 0.1$ µM), Pro are the major component of the integrated carbon biomass. In the layers where $NO_3 + NO_2$ concentration > 0.1 µM, the Peu constitute the major component. In atoll-lagoonal and ocean boarding waters, when $NO_3 + NO_2 \approx 0.1$ µM, small concentrations of NH_4^+ appear to promote Syn abundance. Nevertheless in lagoonal waters additional factors such as the average depth of the lagoon and nitrogen fixation must be taken into account.

INTRODUCTION

The picophytoplankton, as defined by Sieburth *et al.* (1978), constitute a large part of the phytoplankton biomass found in large areas of the world ocean.

Changes in phytoplankton size structure are directly associated to the availability of nitrogen nutrients. Le Bouteiller *et al.* (1992) demonstrated in the intertropical Atlantic and Pacific oceans, that in the non nitrate depleted layer, chl a >1 µm predominates and that the chl a <1 µm predominates in nitrate depleted layers. The concentration at which the switch of chlorophyll a occurs is 0.1 µM ($NO_3 + NO_2$). Below this concentration, the size fraction < 1µm is dominant, above this concentration the size fraction > 1µm become dominant.

Other studies reported that the picophytoplankton is constituted of 3 fluorescent groups the prochlorophytes (*Prochlorococcus marinus*), cyanobacteria (*Synechococcus* spp.) and the picoeukaryotes (that belong to numerous algal families). The ubiquitous offshore distribution of these populations is now well described (Murphy and Hagen 1985, Olson *et al* 1990$_{a,b}$, Campbell and Vaulot 1993, Shimada *et al.* 1993, Ishizaka *et al.* 1994, Partensky *et al.* 1996, Blanchot and Rodier, 1996). In both Atlantic and Pacific oceans the nutrient depleted areas are largely dominated in cell number, pigment concentration and carbon biomass by the *Prochlorococcus* (Campbell *et al.* 1994; Blanchot and Rodier 1996; Partensky *et al.* 1996). The presence of nutrients in the well illuminated layers induce the growth of the other groups (Blanchot and Rodier 1996; Partensky *et al.* 1996), even if the input of nutrients is transient (Blanchot *et al.* 1992). In offshore biotopes the apparent dominance of *Synechococcus* is exceptional, despite its reported presence (Partensky *et al.* 1996). In contrast the significant dominance of *Synechococcus* both in cell abundance and in biomass tends to be the rule in lagoonal coral environments (Blanchot *et al.* 1989; Charpy *et al.* 1992; Charpy and Blanchot 1996$_{a,b}$).

In the present work, a comparison of different sites is conducted: offshore (west and central equatorial Pacific) and coastal inner and boarding waters (Tuamotu archipelago, Fiji archipelago) of open and closed atolls is used to describe the variations of the picophytoplanktonic community structure in relation to the availability of nitrogenous nutrients.

MATERIALS AND METHODS

Observations were made during two cruises in the western and central Pacific (SURTROPAC 17, FLUPAC), aboard R/V « Le Noroit » and « l'Atalante ». In the Fiji archipelago, observations were made, in the lagoon of the Great Astrolabe Reef (18°45'S - 178°30'E) and in the surrounding ocean, during the ASTRO cruise, aboard R/V « L'Alis ». In Tuamotu archipelago, the Takapoto experiment was conducted at the EVAAM station of Takapoto (14°30'S - 145°20'W) and at 9 atolls (Haraiki, Hikueru, Hiti, Kauehi, Marokau, Nihiriu, Reka-Reka, Taiarao and Tepoto Sud) (Fig. 1).

During offshore cruises, hydrocasts to 1000m were made every degree and every 0.5 degree in the equatorial zone with a Seabird CTD (SBE 9). Water samples from discrete depths were collected with 5 and 12 l acid-cleaned Niskin bottles attached to a rosette sampler. During the ASTRO cruise and Takapoto experiment, water samples from discrete depths were collected with 1.7 l acid-cleaned Niskin bottles attached to a wire. The standard depths are shown in each depth profiles.

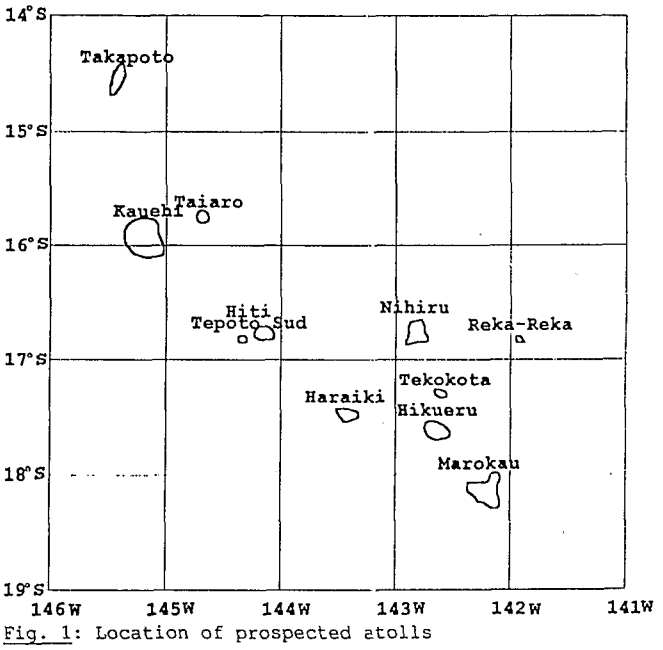

Fig. 1: Location of prospected atolls

Nutrient determinations

During offshore cruises $NO3^-$ + $NO2^-$ analyses were performed immediately on-board with a Technicon Autoanalyzer II, using slight modifications of standard colorimetric methods (Strickland and Parsons, 1972). Additionally, a low level (10 -1500 nM) module was used, according to the high sensitivity method defined by Oudot and Montel (1988). During coastal cruises, dissolved nutrients were determined immediately using the standard techniques described by Strickland and Parsons (1972).

Flow-cytometry

A FACScan (Becton-Dickinson), flow cytometer (FCM) equipped with 488 nm argon laser (power = 15mW) was used for enumeration of photosynthetic picoplankton. The Becton-Dickinson LYSIS2 software was used for data acquisition and analysis. Briefly the FCM was installed in a dark and temperature controlled laboratory. Sea water filtered through GF/F filters was used as sheath fluid. Typically 0.1 ml of sea water was analyzed. Samples were run within 2 hours after sampling as reported by Blanchot and Rodier (1996). For each cell, five variables were recorded on 4-decade logarithmic scales. Cellular fluorescence was always expressed relative to the fluorescence of calibration beads (in arbitrary units, a. u.), by dividing the mean cell fluorescence by the mean bead fluorescence. Before and after running each sample set, flow rate was determined using a known concentration of beads (counted by microscopy), according to Olson *et al.* (1988).

Table 1 : Integrated picophytoplankton carbon biomass and cell abundance in relation to N_n availability. (* Flupac data; *ocean+ = surrounding ocean*). (the respective % of integrated carbon biomass for the different populations are in bracket)

	N_n µM	Biomass gC m^{-2}	Pro gC m^{-2}	Pro cells m^{-2} 10^{10}	Syn gC m^{-2}	Syn cells m^{-2} 10^{10}	Peu gC m^{-2}	Peu cells m^{-2} 10^{10}
offshore								
0S-167E (160m)	N_n<0.1	1.412	0.974 (69%)	1.596	0.014 (1%)	13	0.424(30%)	14
10S-150W (160m)	N_n >3	2.373	0.716 (30%)	1.174	0.065 (3%)	63	1.591 (68%)	51
Coastal								
Takapoto (25m)	N_n ≈0.2 NH_4 ≈0.2	0.523	0.034 (7%)	56	0.285 (55%)	274	0.203 (39%)	7
ocean+ (160m)		1.785	1.059 (59%)	1.737	0.015 (1%)	15	0.711 (40%)	23
Astro. (35m)	N_n ≈0.4 NH_4 ≈0.3	0.396	0.040 (10%)	66	0.243 (61%)	233	0.113 (29%)	4
ocean+ (160m)	N_n ≥0.5 NH_4 ≈0.4	1.160	0.358 (31%)	587	0.196 (17%)	189	0.606 (51%)	19

Cell groups (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) were determined, using their optical properties. Prochlorophytes, referred to as *Prochlorococcus marinus*, were easily discriminated from the picoeukaryotes by their much smaller red fluorescence (RF) and side scatter (SSC). *Synechococcus* spp., have intermediate RF and SSC signals between those of *Prochlorococcus* and picoeukaryotes and were distinguished clearly by their orange fluorescence (OF). Picoeukaryotes always have the largest RF and SSC. In order to estimate numerical abundance, we used the histograms provided by the FACScan analysis system (number versus red fluorescence intensity; Lysis II software). At all coastal stations, the *Prochlorococcus* populations were sufficiently bright to be completely resolved by the FACScan system. At the offshore oligotrophic stations, in the upper layers, the *Prochlorococcus* were too dim to be completely resolved by the apparatus and we estimated the cell abundance by extrapolation described by Blanchot and Rodier (1996). Global estimations of populations parameters were achieved by multiplying the mean value of concerned cellular parameters by the abundance of cells.

Carbon biomass estimates

The conversion factors used for carbon estimates were computed using: 61 fgC for *Prochlorococcus*, 104 fgC for *Synechococcus* and 3110 fgC for picoeukaryotes, respectively. Blanchot and Rodier (1996).

RESULTS

Offshore environments

Located in offshore Nitrogen depleted areas, found at the Equator (167°E), *Prochlorococcus* are two order of magnitude more abundant than the other groups (Table 1). They are numerous from the surface down to 80 m and they decrease slowly with depth. The picoeukaryotes are slightly more abundant in the vicinity of the nitracline (80 m). Their contribution to the biomass become dominant at the base of the photic zone when nutrients are present (Fig. 2). *Synechococcus* remains scarce throughout the whole of the photic layer. In N_n depleted waters, the respective percentage of integrated carbon biomass for the *Prochlorococcus*, *Synechococcus* and the picoeukaryotes are estimated respectively 69%, 1%, and 30%.

Where the nitracline is deep (120 m - 140 m at 14°S - 165°E, Blanchot and Rodier, 1996) the *Prochlorococcus* contributed up to 78% of the integrated carbon biomass (Table 1).

At Equatorial sites where nitrogen was non depleted (150°W: N_n > 1 µM surface), picoeukaryotes and *Synechococcus* were 4 and 5 times more numerous than in the depleted areas. *Prochlorococcus* were less abundant than in the depleted waters but still remained two order of magnitude more abundant than the other groups. Nevertheless, they constitute only a third of the integrated carbon biomass due to their small size. The picoeukaryotes are dominant in biomass from the surface down to the poorly lit layers (Fig. 3).

Depth-profiles of *Prochlorococcus* and picoeukaryotes biomass are parallel and no maximum is evident. Despite the fact that in areas with high N_n, *Synechococcus* are 5 times more numerous than in the depleted areas; they represent a small percentage of the integrated biomass. In high N_n waters, the percentage of integrated carbon biomass contributions made by *Prochlorococcus*, *Synechococcus* and picoeukaryotes were 30%, 3%, and 67%, respectively. Along 165°E, at 11°S where N_n is not depleted, Blanchot and Rodier (1996) reported very similar findings with picoeukaryotes dominating in integrated carbon biomass of 68% (Table 1).

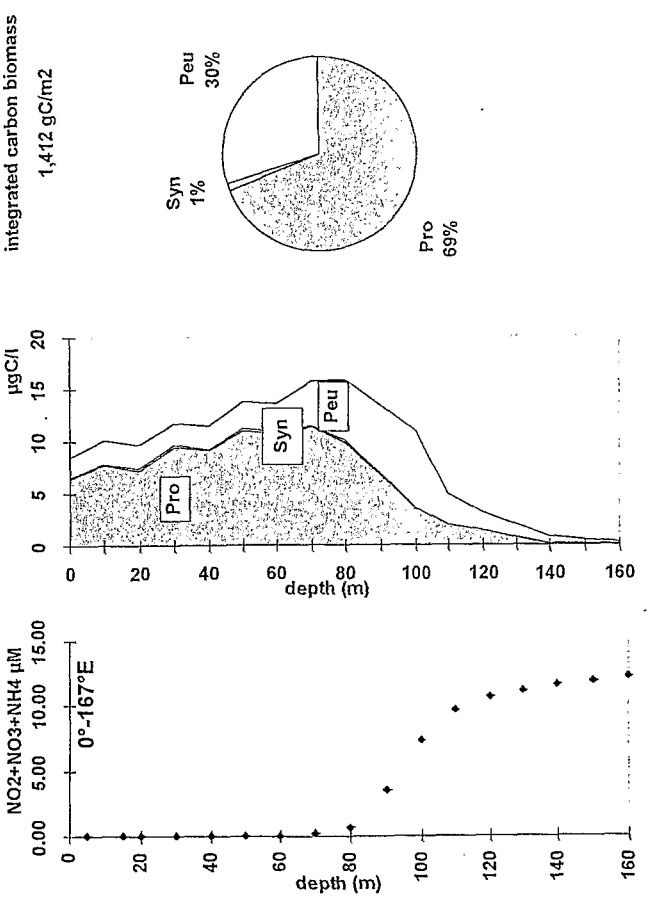

Fig. 2 : Depth profiles of Nitrogen nutrients and carbon biomass of the picophytoplanktonic groups at 167°E -0°).

Table 2: Characteristics of the 9 surveyed atolls, phytoplankton carbon (PkC) and contributions of *Prochlorococcus* (Pro), *Synechococcus* (Syn) and picoeukaryotes (Peu) to PkC. S = lagoon surface area, NP = number of passages, EAD = Estimated average depth

Atoll	Latitude	Longitude	S km^2	NP	EAD m	NH_4 µM	$NO_2 + NO_3$ µM	N_n µM	PkC µg l^{-1}	% Pro	% Syn	% Peu
Kauehi	15°50'S	145°09'W	320	1	50	0.08	0.02	0.10	10.4	49	32	19
Taiaro	15°45'S	144°38'	9	0	15	0.11	0.04	0.15	13.2	6	35	59
Tepoto Sud	16°44'S	144°17'W	2	1	5	0.2	0.43	0.63	35.3	2	82	16
Hiti	16°43'S	144°17'W	24	0	10	0.07	0.08	0.15	25.2	68	27	5
Haraiki	17°28'S	143°26'W	11	1	10	0.16	0.28	0.44	20.9	4	95	2
Hikueru	17°35'S	142°38'W	79	0	25	0.05	0.02	0.07	7.2	15	64	21
Marokau	18°03'S	142°16'W	220	1	30	0.09	0.02	0.11	10.3	52	40	8
Reka-Reka	16°50'S	141°55'W	3	0	1	0.35	0.2	0.55	2.1	3	35	62
Nihiru	16°41'S	142°50'W	88	0	20	0.07	0.08	0.15	9.3	37	56	8

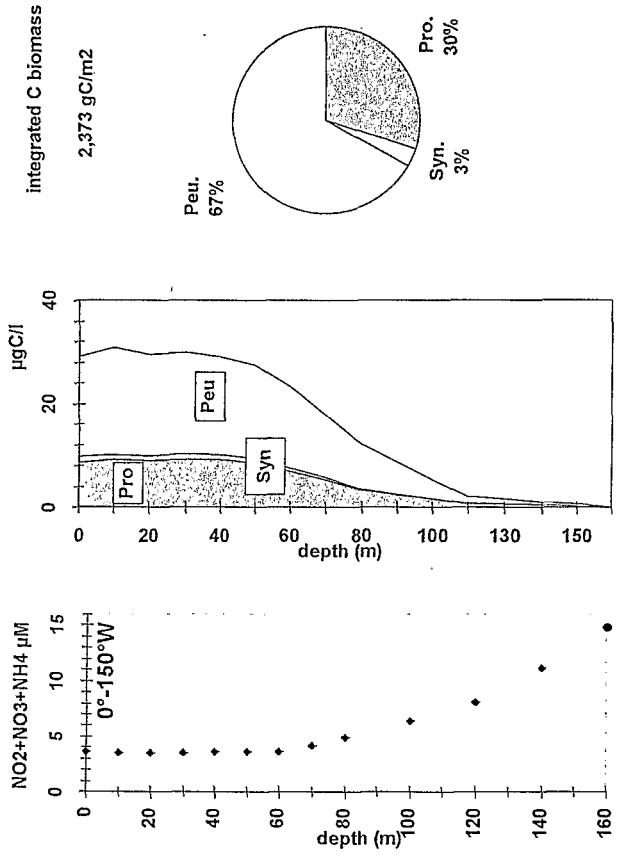

Fig. 3 : Depth profiles of Nitrogen nutrients and carbon biomass of the picophytoplanktonic groups at (150°E -0°).

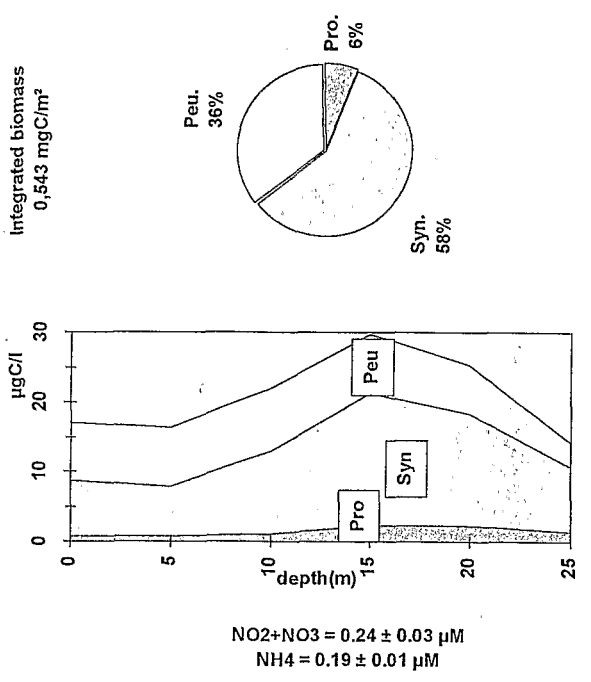

Fig. 4 : Depth profiles of carbon biomass of the picophytoplanktonic groups at the lagoon of Takapoto (145°20'W - 14°30'S).

Coastal environments

In the Coastal environment we distinguish between the inner lagoonal waters and the waters of the surrounding ocean of the closed and open lagoons.

Case 1 Closed lagoon waters not depleted of ammonium.

The inner lagoon waters of Takapoto are not nutrient depleted (N_n= 0.4 µM with NH4 = 0.2 µM). In the closed atoll of Takapoto phytoplankton are heterogeneously distributed. The picoeukaryotes are dominant near the surface, while the *Synechococcus* are dominant in the under layer (Fig. 4). The *Prochlorococcus* are present but scarce throughout the water column. *Synechococcus* exhibit a maximum in carbon biomass at 15 m and is the most dominant group in terms of carbon biomass from 10 m to the bottom, constituting 58% of the integrated carbon biomass (picoeukaryotes 36%), (Fig. 4, Table 1).

The distribution of the picophytoplanktonic cells in the ocean surrounding Takapoto is typical of N_n depleted waters (Fig. 5); *Prochlorococcus* constitute the major group throughout the photic zone (59% of the integrated carbon biomass).

Case 2 Open lagoon waters not depleted of ammonium

The inner waters of Great astrolabe reef's open lagoon are not nutrient depleted (N_n = 0.4 µM with NH_4 = 0.3 µM at surface). The vertical distribution the phytoplankton is homogeneous. *Synechococcus* are the dominant group throughout the whole water column, reaching a maximum in the middle of the water column (Fig. 6). A low level of abundance of *Prochlorococcus* is observed from the surface to the bottom. In open lagoon systems, the respective percentages of integrated carbon biomass for the *Prochlorococcus*, *Synechococcus* and the picoeukaryotes are 10%, 61%, and 29% (Fig. 6, Table 1).

The community structure of the surrounding ocean is heterogeneous. In the upper layers (0 - 60m), the three groups are well defined. Due to the poor photoadaptation of *Synechococcus* this group disappears rapidly with depth. In surrounding waters, the percentage of integrated carbon biomass for the *Prochlorococcus*, *Synechococcus* and the picoeukaryotes are 31%, 17%, and 52% respectively (Fig. 7, Table 1). This unusual distribution could be due to several factors: The high nutrient laden waters promote growth in picoeukaryotes. This high nutrient level could be the result of vertical mixing associated to "island mass effects". In addition, numerous passages and submerged reefs allow for active exchanges of water between the lagoon and the ocean. These exchanges produce an enrichment of *Synechococcus* in the surrounding ocean, and an enrichment of *Prochlorococcus* in the inner waters.

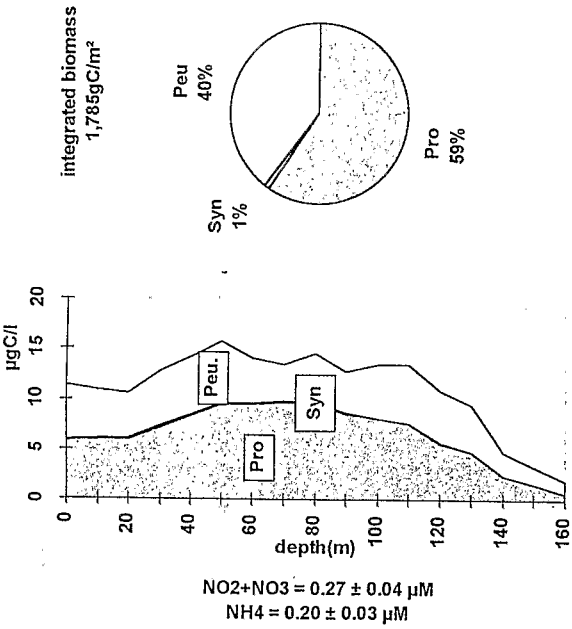

Fig. 5 : Depth profiles of carbon biomass of the picophytoplanktonic groups at the surrounding ocean of Takapoto (145°20'W - 14°30'S).

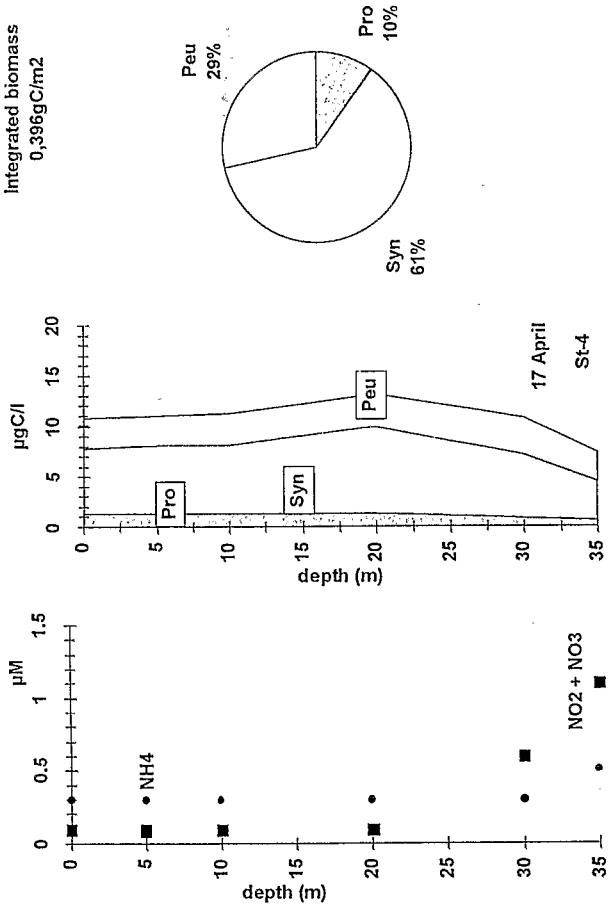

Fig. 6 : Depth profiles of Nitrogen nutrients and carbon biomass of the picophytoplanktonic groups at the lagoon of Great Astrolabe Reef (178°30'E - 18°45'S).

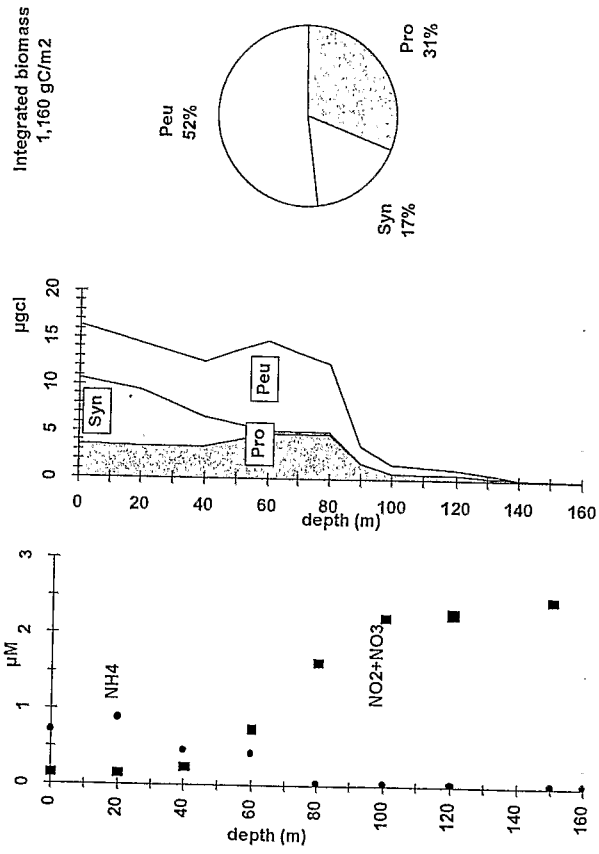

Fig. 7 : Depth profiles of Nitrogen nutrients and carbon biomass of the picophytoplanktonic groups at the surrounding ocean of Great Astrolabe Reef (178°30'E - 18°45'S).

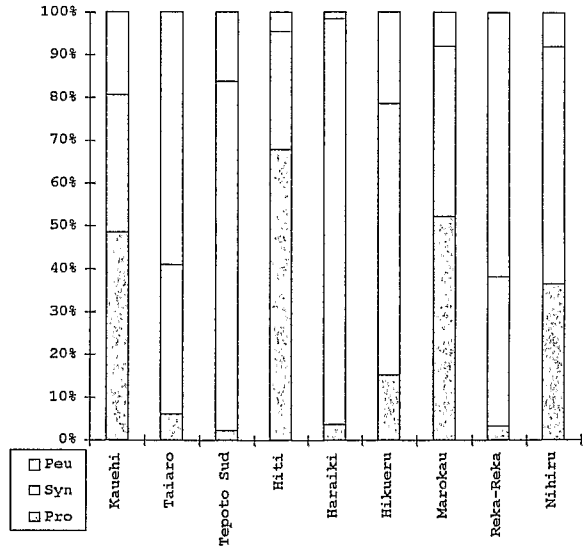

Fig. 8 : Contribution of *Prochlorococcus*, *Synechococcus* and picoeukaryotes to picophytoplankton carbon biomass at 9 atolls (Tuamotu archipelago)

Miscellaneous cases in relation to depth, passage and N_n

To complete our study on the community structure of picophytoplankton in the Tuamotu atoll groups we surveyed 9 atolls, which characteristics appear in Table 2.

Synechococcus are abundant at all atolls. They constitute between 27 % and 95 % of the carbon integrated biomass (Table 2, Fig. 8). Nevertheless *Synechococcus* are not always the dominant group in terms of integrated carbon biomass: for instance in Kauehi and Taiaro *Prochlorococcus* and picoeukaryotes are dominant respectively. When the depth is very shallow ≤ 5 m the dominant group is dependent on the flush of water and no general trend could be observed (Reka-Reka, Hiti).

DISCUSSION

Irrespective of the environment (offshore or coastal) the prokaryotic cells were always dominant in terms of cell abundance. However dominance in terms of integrated carbon biomass depends on nitrogen nutrients availability.

The relative contributions of picophytoplanktonic groups are largely driven by the N_n availability. The offshore depleted layers are the realm of *Prochlorococcus*. The ability of small cells to grow at very low nutrient concentrations are sustained by theoretical considerations. For example Chisholm (1992) indicated that even at the very low concentrations (N_n = 5 nM), a cell radius of ≈ 0.35 µm could potentially grow at a rate of 1 day^{-1}. This very low concentration is under our concentration threshold of 0.1 µM. For N_n concentration less than 0.1 µM, we routinely counted 2 10^5 *Prochlorococcus* ml^{-}. When $NH_4 \geq 0.1$ µM with $NO_2 + NO_3 < 0.1$ µM, the abundance of *Synechococcus* increased. In offshore areas, the dominance in carbon biomass of Prokaryotic cells disappears as soon as the N_n are not depleted i.e. $NO_2 + NO_3 > 0.1$ µM. As reported by Blanchot *et al.* (1992), Le Bouteiller *et al.* (1992), the threshold nutrients $NO_2 + NO_3 \approx 0.1$µM share the water column in two layers. The depleted one, where the size structure of chlorophyll and the biomass of picophytoplankton is dominated by the fraction < 1µm (prokaryotic cells). The second one, when $N_n \geq 0.1$ µM, where chlorophyll and carbon biomasses are dominated by cells > 1µm (eukaryotic cells). The overall dominance of *Synechococcus* in lagoonal waters irrespective of the concentration of nutrients, raises the question: what are the underlying reasons for such a deviation of the general rule reported previously? Several years ago the use of an epifluorescent microscope first allowed us to report on the abundance of *Synechococcus*.

Whatever the environment (offshore or coastal) the prokaryotic cells were always dominant in terms of cell type in the inner lagoons of Tikehau and Takapoto (Blanchot *et al.* 1989, Charpy *et al.* 1992). The use of a flow-cytometer allowed us to count *Prochlorococcus* and reconfirmed the dominance of *Synechococcus* (Charpy and Blanchot 1996, Blanchot 1996). The dominance of *Synechococcus* is promoted by the lagoonal averaged depth 5-25 m. When the averaged depth is shallow (< 5m) the flush of water, does not allow the development of a great *Synechococcus* abundance. In deep lagoons, where the average depth is >25m, the increase of *Prochlorococcus* and picoeukaryotes which can grow at lower light levels is promoted. The abundance of *Synechococcus* begins to decrease below 15-20 m at both Takapoto and Great Astrolabe Reef lagoons (Fig. 3, 4). When the average depth is greater, the deeper layer is dominated in carbon biomass by the other groups. We report that the small concentration of NH_4 appears to promote the increase of *Synechococcus* abundance. We also found, that in low nutrient waters of Hikueru (Table 2) the *Synechococcus* dominate the integrated carbon biomass (64%). One hypothesis is that nitrogen fixation could promote growth in *Synechococcus*. The *Synechococcus* are well known to have nitrogenase activity (Rippka and Waterbury 1977, Grobbelaar *et al.* 1986; Mitsui *et al.* 1986, Huang *et al.* 1988). This activity occurs in the lagoon of Tikehau, where nitrogen fixation in the water column supplies 44 mg N m^{-2} day^{-1} (Charpy-Roubaud *et al.* this volume). Nutrient availability is known to play a vital role in community structure, yet other limiting factors may be of significance. For instance, Martin and Fitzwater (1988) established that iron is a limiting factor in the ocean In response to iron enrichment the phytoplankton community change from nitrogenous nutrition Price *et al.* (1991). Some populations are capable of adaptation. In iron-deficient waters, *Synechococcus* produce different ferric-specific chelator which serves to increase the association of iron to the surface of cells (Wilhelm and trick, 1994). Other micronutrients are required in addition to nitrogen fixation such as molybdenum, vanadium and iron (Zehr, 1992). This information led us to conclude that other studies must be undertaken to refine our knowledge on the picophytoplankton community structure.

Nevertheless, it is reinstated that *Synechococcus* form the most significant group in terms of abundance and that a high percentage of this group constitutes the integrated carbon biomass found in the lagoons. The medium averaged depth (<25m,) small N_n concentrations and the nitrogenase activity seem to promote the observed *Synechococcus* growth. Additional studies will be useful to understand the respective influences of other fixing (benthic) cyanobacteria after being recycled. The influence of benthic fixation is likely to be affected by the retention time of inner waters. Other studies such as the role of trace metal regimes could also play a major role, but to our knowledge, no study is available in coral lagoons at present.

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